HIGH TITER RECOMBINANT AAV VECTOR PRODUCTION IN ADHERENT AND SUSPENSION CELLS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/872,523, filed on Aug. 30, 2013, which is hereby incorporated by reference in its entirety.

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Aug. 28, 2014, is named A-1839-WO-PCT-SeqList082814_ST25.txt and is 2 kilobytes in size.

[0003] Throughout this application various publications are referenced within parentheses or brackets. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0004] The present invention is directed to the field of recombinant viral vector production.

2. Discussion of the Related Art

[0005] Adeno-associated virus (AAV) systems have been used for gene delivery to mammalian cells. AAV is generally considered a good choice for gene delivery because it has not been associated with any human or animal disease and does not appear to alter the biological properties of the host cell upon integration. AAV, which belongs to the genus Dependovirus, is a helper-dependent DNA parvovirus. Thus, in order for effective AAV virion production to occur, the host cell must also be infected with an unrelated helper virus, either adenovirus (Ad), a herpesvirus (HSV), or vaccinia virus. The helper virus supplies accessory functions that are necessary for most steps in AAV replication. In the absence of such infection, AAV establishes a latent state by insertion of its genome into a host cell chromosome. Subsequent infection by a helper virus rescues the integrated copy which can then replicate to produce infectious viral progeny. AAV has a wide host range and is able to replicate in cells from any species so long as there is also a successful infection of such cells with a suitable helper virus. For example, human AAV will replicate in canine cells co-infected with a canine adenovirus. For a review of AAV, see, e.g., Berns & Bohenzky, Advances in Virus Research 32:243-307 (Academic Press, Inc. 1987).

[0006] The AAV genome is composed of a linear single-stranded DNA molecule which contains 4681 bases (Berns & Bohenzky, supra). The genome includes inverted terminal repeats (ITRs) at each end which function in cis as origins of DNA replication and as packaging signals for the virus. The ITRs are approximately 145 bp in length. Inverted terminal repeats flank the unique coding nucleotide sequences for the non-structural replication (Rep) proteins and the structural (VP) proteins. The VP proteins (VP1, -2 and -3) form the capsid. The terminal 145 nucleotides are self-complementary and are organized so that an energetically stable intramolecular duplex forming a T-shaped hairpin may be formed. These hairpin structures function as an origin for viral DNA replication, serving as primers for the cellular DNA polymerase complex.

[0007] The internal nonrepeated portion of the genome includes two large open reading frames, known as the AAV rep and cap regions, respectively. These regions code for the viral proteins involved in replication and packaging of the virion. In particular, a family of at least four viral proteins are synthesized from the AAV rep region, Rep 78, Rep 68, Rep 52 and Rep 40, named according to their apparent molecular weight. Following wild type AAV infection in mammalian cells the Rep genes (i.e. Rep78 and Rep52) are expressed from the P5 promoter and the P19 promotor, respectively and both Rep proteins have a function in the replication of the viral genome. A splicing event in the Rep ORF results in the expression of actually four Rep proteins (i.e. Rep78, Rep68, Rep52 and Rep40). However, it has been shown that the unspliced mRNA, encoding Rep78 and Rep52 proteins, in mammalian cells are sufficient for AAV vector production. Also in insect cells the Rep78 and Rep52 proteins suffice for AAV vector production.

[0008] The AAV cap region encodes at least three proteins, VP1, VP2 and VP3. For a detailed description of the AAV genome, see, e.g., Muzyczka, Current Topics in Microbiol. and Immunol. 158:97-129 (1992). For descriptions of the construction of recombinant AAV virions see, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Numbers WO 92/01070 (published 23 Jan. 1992) and WO 93/03769 (published 4 Mar. 1993); Lebkowski et al., Molec. Cell. Biol. 8:3988-3996 (1988); Vincent et al., Vaccines 90 (Cold Spring Harbor Laboratory Press 1990); Carter, Current Opinion in Biotechnology 3:533-539 (1992); Muzyczka, Current Topics in Microbiol. and Immunol. 158:97-129 (1992); Kotin, Human Gene Therapy 5:793-801 (1994).

[0009] It is possible to make a mammalian cell line stably expressing AAV rep and AAV cap proteins, but it was also reported that AAV rep protein was toxic to the cells. A majority of rAAV vector production is still done by transient transfection. In addition, transient transfection offers the ease of AAV serotype selection by changing DNA constructs compared to the use of stably transfected cell lines.

[0010] Consequently, most on temporary recombinant AAV (rAAV) virion production involves co-transfection of a host cell with an AAV vector plasmid usually containing one or more transgenes flanked by AAV ITRs, and a construct which provides AAV helper functions (e.g., rep and cap) to complement functions missing from the AAV vector plasmid. In this manner, the host cell is capable of expressing the AAV proteins necessary for AAV replication and packaging. To provide accessory functions, the host cell is then be transfected with a plasmid having accessory function or infected with a helper virus, typically an infectious adenovirus (e.g., type 2 or 5), or herpesvirus.

[0011] More particularly, AAV vector plasmids can be engineered to contain a functionally relevant nucleotide sequence of interest (e.g., a selected gene, antisense nucleic acid molecule, ribozyme, or the like) that is flanked by AAV ITRs which provide for AAV replication and packaging functions. After an AAV helper plasmid and an AAV vector plasmid bearing the nucleotide sequence are introduced into the host cell by transient transfection, the accessory function can be provided either by transfecting the cells with a plasmid with accessory genes or by infecting cells with a helper virus, most typically an adenovirus, which, among